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Army Biological Labs ltr dtd 6 Aug 1968

TRANSLATION NO. T591

[redacted]

DATE: 1 July 1968

AD851146

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Translated by the
Translating Unit
Library Branch
Division of Research Services
National Institutes of Health
Bethesda, Maryland

No. 12-15-65

From the Russian for
Dr. William D. Hann

Vop Virus
No. 2, 165-167, 1965

Study of the variability of the tick-borne virus of encephalitis. Report I. Long cultivation of viruses of the tick-borne encephalitis group in renal-cell culture of pig embryo and in chick embryos.

by

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In the literature there are data on the adaptation variability of the arbor viruses through long cultivation in different cell cultures and in growing chick embryos [2-5].

The purpose of the present investigation has been a study of the properties of several strains of viruses of the group of tick-borne encephalitis in the process of long cultivation in chick embryos as well as in a culture of the renal cells of pig embryo. Considering the possible importance of the incubation temperature of the inoculated cultures to the selection of the variants, the passage of the strains under study in a culture of pig-embryo renal cells has been done at 33° and 40°.

Materials and methods

Investigations have been carried out with strains of tick-borne encephalitis virus Pan and Ix-10, and with the I-40 virus strain of Scotch encephalitis.

Inoculation of the cultures with a passage of the viruses has been done on the 3d to 4th day after sowing of the cells when a uniform layer has formed. The virus has been introduced into the test tubes with cell cultures in dilutions of 10^{-1} to 10^{-6}, prepared on medium No 199 with 10% bovine serum. For every dilution of virus, 4 test tubes each have been used. The inoculated cultures have been

incubated at 33° and 40°. Degeneration of cells, as a rule, has set in on the 4th to 5th day at 33° and on the 2d to 4th day at 40°. In this period, selection has been made of the test tubes of virus-containing liquid, which had been inoculated with the highest dilution of the virus that produced degeneration of the cultures. This material has been used for subsequent inoculation of fresh cultures.

The aptitude of the viruses to form plaques has been determined by the method described by us earlier [1]. For inoculation, 9-day chick embryos have been employed. Inoculation of the embryos has been done into the yolk sac with 0.25 ml of a suspension taken in a dilution of 10^{-4} to 10^{-5}, prepared from 3 embryos of the preceding passage. The inoculated embryos have been incubated at 36° for 72 hours.

Results

Three lines of passages have been made with each of the strains investigated: 70 passages on culture of pig-embryo renal cells at 33°, 60 passages on the same culture but t 40°, and 120 passages in chick embryos.

Considering the high sensitivity of mice to tick-borne encephalitis virus, as well as the stability of the neuropathogenic properties of this group of viruses, we have selected as the principal criterion of evaluation of the change of the pathogenic properties of the variants obtained the pathogenicity of the viruses for mice, with different methods of inoculating them.

For this purpose the virus-containing material of the different passages has been titrated on white mice by inoculation into the brain, intraperitoneally, subcutaneously, and per os. It has been demonstrated that the pathogenicity of the viruses at intra-cerebral introduction into white mice of all the strains studied has remained without changes both at passage in a culture of pig-embryo renal cells at 33° and 40° and at passage in developing chick embryos.

A certain reduction has been observed of the infectuosity of the variants obtained of viruses when peripheral methods of introduction into the mice were used. However, it has been insignificant and has fluctuated from passage to passage.

Besides the pathogenicity for mice, we have studied the cytopathic effect of these strains on cultures of SOTs, HEp-2, and L, and on chick fibroblasts. Data on the destructive effect of the viruses are cited in the table. As seen from the materials presented, a distinct cytopathic effect has been noted of all the strains on a culture of pig-embryo renal cells, the titers of the viruses, according

to the destructive effect on this culture, having corresponded to the titers obtained at intracerebral inoculation of the mice.

Certain variants obtained as a result of the adaptation to renal cells of pig embryo have acquired the capacity to destroy SOTs cells. This property has been particularly clearly expressed in the I-40 strain, adapted to different conditions, and in the Pan strain that has been passed in chick embryos. The Pan strain, adapted to culture of pig-embryo renal cells at 40° and 33°, as well as the Ix-10 strain, passaged at 33°, has provoked a partial destruction of the culture of the SOTs cells. The Ix-10 strain, adapted to the culture of pig-embryo renal cells at 40° and to chick embryos, has not possessed this property.

Strains Ix-10, Pan, and I-40, passaged in a culture of pig-embryo renal cells at 40° and 33°, have shown a clear cytopathic effect on a culture of cells of chick fibroblasts. Viruses, cultured in chick embryos, have not possessed this property, except for the Pan strain, which provoked partial destruction of culture of the cells of chick fibroblasts.

The variants obtained of the viruses have shown no cytopathic effect on L and HEp-2 cells.

The Pan and I-40 strains, both of ixodids and their variants, have formed negative spots in the culture of pig-embryo renal cells. The plaques, as a rule, have appeared on the 3d day, have had a rounded form, and have attained a diameter of 2 to 4 mm. The Ix-10 strain of plaques have not formed.

In the process of adaptation to different conditions of cultivation, the aptitude of the viruses to form plaques has not been changed. The form and dimensions of the plaques of the variants obtained of the viruses have been identical to the initial. Strain I-40, adapted to culture of pig-embryo renal cells at 33°, has constituted the exception. This variant of the virus came to form very small plaques (diameter 0.5 mm); however, its pathogenicity to mice has not been distinguished from the pathogenicity of the strains that formed large plaques.

For the SOTs cells, not one of the initial strains of plaques have formed. Nevertheless, their variants, obtained as a result of long passaging in cultures of pig-embryo renal cells at 33° (strain I-40), at 40° (strain Pan and I-40), and in developing chick embryos (strain I-40), have acquired the aptitude to form plaques in cultures of SOTs cells. According to the periods of the manifestation

and the dimensions of the plaque for SOTs cells, they have not been distinguishable from the plaques formed by these viruses on a culture of pig-embryo renal cells.

Conclusions

1. As a result of the prolonged passaging in a culture of pig-embryo renal cells at 33° (70 passages) and at 40° (80 passages), as well as in developing chick embryos (120 passages) of the viruses of the tick-borne encephalitis group (strains Ix-10, Pan, and I-40), the neuropathogenic properties of these strains for white mice have not been changed.

2. As a result of the adaptation, certain strains have acquired the aptitude to destroy SOTs cells and fibroblasts of chick embryos.

3. Pan and I-40 strains, both the initial and the variants obtained, have possessed the property to form plaques in culture of pig-embryo renal cells. Variants of the viruses, obtained at growing in cultures of pig-embryo renal cells at 40° (Pan and I-40), at 33°, and in thick embryos (I-40), have acquired the capacity to form plaques in cultures of SOTs cells.

Bibliography

ЛИТЕРАТУРА

1. Анджапаридзе О. Г., Степанова Л. Г. Вопр. вирусол., 1961, № 4. стр. 404. — 2. Кравченко А. Т., Васильев Б. Н. Там же, № 1, стр. 10. — 3. Berge T., Banks I., Tigertt W., Am. J. Hyg., 1961, v. 73, p. 209. — 4. Bhatt P., Work T., Proc. Soc. exp. Biol. (N. Y.), 1957, v. 96. p. 213. — 5. Molnár E., Acta microbiol. Acad. Sci. hung., 1959, т. 6, стр. 23.

Cytopathic and plaque-forming properties of the viruses studied of the group of tick-borne encephalitis, adapted to different conditions of culturing

Criterion of Evaluation	Culture	STRAINS Ix-10	Pan	I-40
Cytopathic effect	Pig-embryo kidney			
	Chick-Fibroblasts			
	SOTs			

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Cytopathic and plaque-forming properties of the viruses studied of the group of tick-borne encephalitis, adapted to different conditions of culturing

Criterion of Evaluation	Culture	STRAINS Ix-10	Pan	I-40
Cytopathic effect	Pig-embryo kidney			
	Chick-Fibroblasts			